THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.

110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

MAY 10 1974

Application for Research Grant

(Use extra pages as needed)

Date:

1. Principal Investigator (give title and degrees):
   L.G. Abood, Professor of Biochemistry and Brain Research, Ph.D.
   K. Lowy, Professor of Brain Research, M.D. (co-principal investigator)

2. Institution & address:
   University of Rochester Medical Center
   Rochester, New York 14642

3. Department(s) where research will be done or collaboration provided:
   Center for Brain Research

4. Short title of study:
   Behavioral effects of nicotine and piperidine using a computer-controlled program.

5. Proposed starting date: January 1, 1975

6. Estimated time to complete: December 31, 1977

7. Brief description of specific research aims:

The overall objective of this program is to determine the behavioral effects of nicotine and piperidine in cats with a view towards a better understanding of the involvement of cholinergic systems in specific behavioral parameters.

1) Determine the effect of both nicotine and piperidine on a number of quantifiable psychophysical parameters associated with computer-controlled behavioral paradigm in cats.

2) Determine the extent to which nicotine and piperidine will reverse the alterations in the psychophysical patterns produced by an anticholinergic psychotomimetic glycolate, N-methyl 4-piperidyl cyclobutylphenyl glycolate.

3) If some distinct psychophysical parameters are observed with nicotine, conversely, we plan to test a variety of glycolate esters for their ability to reverse such effects.

8. Brief statement of working hypothesis:

Our hypothesis is that there are poorly understood nicotinic systems within the brain that are involved in many aspects of brain function. With the use of nicotinic drugs and their antagonists and making use of more sophisticated techniques for measuring behavior we hope to determine certain behavioral manifestations of such systems. Of particular significance is the fact that piperidine, a known nicotinic drug, is an effective tranquilizer and, possibly, an endogenous psychochemical or neurotransmitter.

9. Details of experimental design and procedures (append extra pages as necessary)

Background

As part of an extensive program to investigate the structure-activity relationships and mechanisms of actions of a group of anticholinergic psychotomimetic glycolate esters (1,2) it was observed that certain cholinergic agents acted as effective antagonists to the agents. Among the most effective agents in this group were physostigmine, tetrahydroaminoacridine, and piperidine (1,3). All three, which were nicotinic as well as muscarinic agents, reversed both the peripheral anticholinergic and psychotomimetic actions of the glycolate esters. The most interesting of the three was piperidine because of its extremely low toxicity and since it was found to have a pronounced tranquilizing effect in animals and man (4).

An extensive clinical trial with piperidine was then undertaken to determine its efficacy as a tranquilizing drug. It was first tested in a large group of psychotic patients (2-4 g/day orally) in a maximal security division of a state hospital, and the results were quite dramatic (4). Unfortunately when tested on another group of psychotic patients at the same hospital and elsewhere, the drug had to be discontinued because of severe nausea and vomiting in 1/3 of the patients. It was later determined that 75% of the first group of patients (maximal security) were heavy smokers and, consequently, had developed a considerable tolerance to the nicotinic action of piperidine. Nevertheless, because of this serious side effect, interest in piperidine as a potential psychotherapeutic agent rapidly subsided.

There are, however, other reasons for an interest in piperidine. Its presence in mammalian brains has been known for some time (5,6), and, recently, has stimulated more interest (7,9). Dolezalwa et al (9) have demonstrated that piperidine may have a physiological role in the molluscan central nervous system, insofar as endogenous piperidine tends to accumulate in the central ganglia of snails during hibernation. They also showed that minute amounts (10^{-14} moles) injected microionphoretically into the ganglionic neurons mimicked the threshold inhibitory

effect of acetylcholine on some cells and had a cholinolytic action on the other cells. Such responses are reminiscent of the action of the nicotinic-muscarinic action of acetylcholine in peripheral cholinergic systems.

Previous work done by us

We have been interested in developing psychophysical measurements which may provide more accurate quantitative and qualitative evaluation of more complex behavioral patterns that may assist in the evaluation of various psychotomimetic and other psychotropic drugs. An anticholinergic psychotomimetic agent was examined for its behavioral effects on cats trained to press a lever whose location corresponded to one of two sound sources. The cats were trained to lick a protruding sponge in dim light which then caused the main light to turn on and an auditory signal to be emitted from either side of a panel in the chamber. Any lever response terminated the trial. A food reward was given only if the cat pressed a lever on the same side as the sound signal. A new trial cycle began when the cat licked the sponge. A computer program controlled the experiment, stored the experimental data, and permitted an analysis of various psychophysical parameters, such as ability to localize an auditory cue, threshold of sound intensity, rate of trial onset, and lateral tendency. Doses of 10 - 20 µg/kg, N-methyl 4-piperidylcyclobutylphenyl glycolate (CBG) reduced the number of responses, and tended to lower the relative time spent in the light period. However, at lower doses CBG produced a marked increase in some cats in total number of trials. Higher doses of scopolamine also reduced total trials, but less consistently. An increase of the number of responses was not observed. A number of animals exhibited a lateral preference for either the right or left lever. CBG, but not scopolamine, markedly shifted this lateral tendency in some cats. On the basis of these studies a number of correlations could be made with respect to the central effects of the glycolate esters in man.

Proposed Research Program

Our first objective is to determine the effects of nicotine and piperidine on cats, utilizing the computer-controlled program used in a previous study (16), and to be described in detail below. After a baseline has been established with a number of quantifiable psychophysical parameters, an attempt will be made to revise or block any effects with the centrally active anticholinergic glycolate esters. The converse study will also be undertaken: nicotine and piperidine will be used to reverse or block the behavioral effects of the anticholinergic glycolate esters.

Detailed description of behavioral program: A scheme of the behavioral program with the sequence of events is presented in figure 1. The sound-proofed testing chamber contained omnidirectional levers on either side of a sponge, all three being located in such a fashion that the cat could readily nudge the sponge with its nose from a standard position and subsequently press either of 2 levers. A schematic diagram (front) of the sound-proofed chamber showing the relative positions of the speakers, levers, and sponge is presented in figure 2.

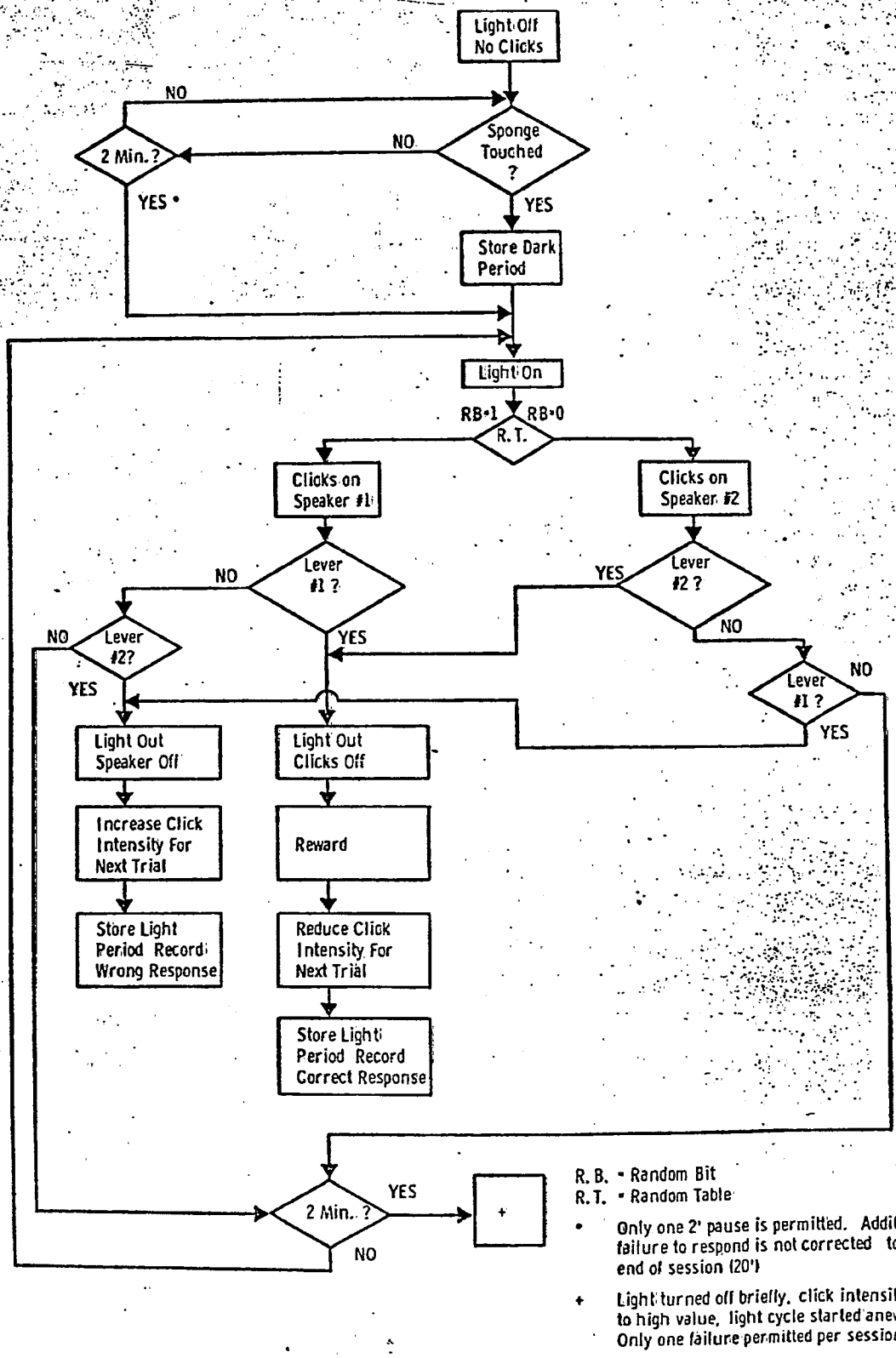


Figure 1

Detailed scheme of behavioral program controlled by LINC 8 Computer

The sequence of events, sound intensity, and sound source were controlled by a LINC-8 computer. The data, which were stored on magnetic tape, included the sequence and intensity of left and right clicks, the correct and wrong responses, and the duration of the light or dark periods. In addition, the tape also contained all necessary bookkeeping information: identification of animal, date, type and dosage of drug, as well as information of specific interest (e.g., erratic performance and pupillary reaction).

A single trial consisted of two phases. During the dark period (with only a small pilot light illuminating the feeding area), no click stimulation was presented, and the levers were inoperative. To start the light period, the cat had to touch a centrally located sponge connected to a contact-sensing device (drinkometer). This maneuver turned on the ceiling light and started a click series on one of the randomly selected speakers. The light period was terminated by the cat's touching one of the levers, which turned off the ceiling light and the clicks. Only a correct (homolateral to the speaker) response was rewarded by a small cup of milk. The animal consumed the reward during the dark period.

The initial training in selecting left versus right lever in response to loud clicks and operating the sponge was done manually, requiring 3 to 4 weeks. The training procedure was subsequently performed by a computer program, which also controlled the intensity of the clicks of 1 msec duration, presented continuously at a rate of 10/sec. The pulse generator was so designed that the output voltage was determined by the duration of pulses generated by the computer in discrete steps and applied to the generator within its linear range (10). Calibration by means of a microphone and oscilloscope showed that a single step very closely corresponded to a change in sound intensity of 1 db. The normal cat's threshold was found to be only a few db below that of humans.

This arrangement permitted on-line control of intensity according to a "titration" method (11). Following a correct response, the click intensity was reduced; a wrong response caused an increase in click intensity. It was soon observed, however, that after a series of fortuitous correct responses the sound intensity became so low that it remained below threshold for a substantial part of the session. This undesirable contingency was successfully eliminated by increasing sound intensity by two steps after a wrong response, an arrangement adopted for all subsequent tests.

Pharmacology: All of the psychopharmacological studies will be carried out in adult cats, both male and female. Cats will be injected 1 ml of one of the following: saline, 10-25 mg/kg piperidine HCl, 0.05-0.20 mg/kg nicotine sulfate; 0.010-0.050 mg/kg N-methyl 4-piperidylbenzilate. Throughout the testing period (at least 2 years), the cats will be maintained at their normal body weight, allowed access to water for only an hour daily, and given a vitamin supplement as needed. They will be tested 5 times/week.

Reversal of nicotinic effects with the anticholinergic glycolate esters

One of the important problems in neuropharmacology is the extent to which nicotinic cholinergic systems are operative in the central nervous system. There are some neurons of the central nervous system, such as the Renshaw cells in the spinal cord (12), thalamus (13), corpus stratum (14), and medulla oblongata-pons (15), which appear to be nicotinic. Although there are some drugs, such as ganglionic blocking agents and certain cholinolytics which will antagonize some of the control effects of nicotine, there is a due need for more specific, potent agents.

As part of a program to investigate the structure-activity relationships of centrally active anticholinergic agents, literally hundreds of glycolate and related esters were synthesized by many collaborative investigators [see (1) and (2) for reviews]. We hope to test a few representative agents of this series for possible central 'antinicotinic' action. Included in this group will be a piperizanol, piperidinol, quinuclinol, and pyrollizadinol benzilate. All of these agents are presently in our possession. Some of these agents have been suspected of having central antinicotinic action (1).

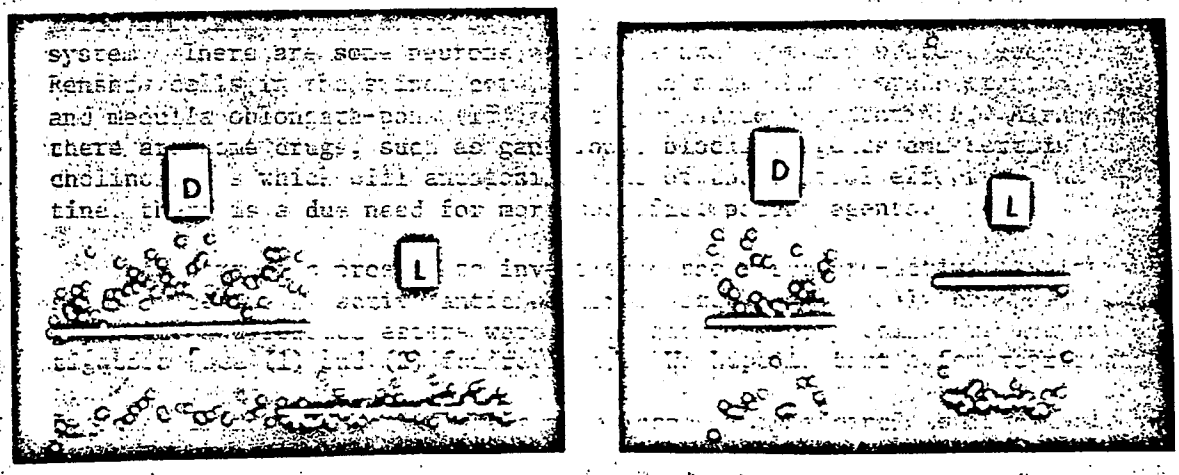
Studies to date with nicotine

During the past few months the effect of nicotine on various psychophysical parameters has been investigated in 6 trained cats. The dose used was 0.1 mg/kg and the cats were tested in the computer-controlled behavioral paradigm as previously described.

The preliminary findings were as follows: 1) Some of the cats made an increased number of trials during the session, while the level of performance tended to be more constant. (Normally the number of trials tends to fluctuate somewhat from session to session.) 2) One of the interesting observations is the effect of nicotine on the relative time spent in the light and dark period during the trials. The dark period corresponds to the interval between a trial termination and onset, while the light interval corresponds to latency between stimulus onset and lever response. Dark period duration can then be used as index of response rate. The effects of nicotine can be seen in figure 2a and 2b, which is an oscillographic display of the relative time intervals automatically analyzed by the computer program. Without drug the dark period was greater than the mean light period; whereas, after a dose of 100 µg/kg nicotine the light period was actually greater than the dark period. The significance of this reversal in time intervals is not entirely known, and, to date, it has been observed in only one animal. It is probably indicative of an increased response rate as borne out by the fact that the number of trials/session was increased in this same cat by nicotine.

Reversal of nicotinic effects with the ...

One of the important problems in ...



a b

Figures 2a and 2b. ...

In each photograph (oscillographic display) is represented ... the scatter of time points for an individual trial during dark (D) and light (L) period; the mean is represented by the line. Figure a is control and b is after 100 mg/kg nicotine.

The preliminary findings were as follows: 1) ... 3) The number of errors, i.e. the number of times the wrong lever was pressed, was unaffected by nicotine. 4) There was no overall effect on laterality, i.e. the preference of cats to press the left or right paw levers was not altered. Here again, with nicotine however, there was less variability in the performance from session to session. 5) There was no change in auditory threshold or in the relative time spent in the dark and light periods. 6) At the doses of nicotine employed there was no evident effect on the cats' appetite for food.

These preliminary findings suggest that at a dose of 0.1 mg/kg nicotine does not impair the cat's learned performance, but, in some instances, tends to make performance more constant. It will be interesting to determine how long-range chronic administration of nicotine influences any of the parameters, particularly with regard to their variability.

References

1. Abood, L.G. and Biel, J.H. (1962) Anticholinergic psychotomimetic agents. Int. Rev. Neurobiol. 4, 218-271.

2. Abood, L.G. (1968) The psychotomimetic glycolate esters, In "Drugs Affecting the Central Nervous System". v. 2 A. Burger, Ed. M. Dekker, New York 127-167.

3. Abood, L.G., Rinaldi, F., Eagleton, V. (1961) Distribution of piperidine in the brain and its possible significance in behavior. Nature 191, 201-202.

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10. Lowy, K. and Ota, L. (1968) Pulse generation with external amplitude controlled by computer. Electroenceph. Clin. Neurophysiol. 25, 389-391.

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14. Herz, A. and Zreglgansberger, W. (1968) The influence of microelectrophoretically applied biogenicamines, cholinomimetics, and procaine on synaptic excitation in the corpus stratum. Int. J. Neuropharmacol. 7, 221-230.

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16. Lowy, Karl, Weiss, Bernard, and Abood, Leo G. (1974, in press) Influence of an anticholinergic psychotomimetic agent on behavior in cats controlled by an auditory stimulus. Neuropharmacology.

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The physical facilities are located within the Center for Brain Research which occupies a total of 25,000 sq. ft. of space within the Medical Center. The space specifically allocated to this proposal consists of two behavioral experimental rooms comprising a total of 800 sq. ft. In one of these is housed the LINC-8 and ancillary equipment for on-line studies. A PDP-8 is also available along with the facilities of the Computer Center which are located in another part of the Medical Center. Within the Center for Brain Research are extensive neurophysiological, neurochemical, and neurohistological facilities as well as fully adequate animal quarters and an electronics shop.

11. Additional facilities required:

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

CURRICULUM VITAE

Leo G. Abood

Born:

REDACTED

Education: Ohio State University, B.S. Chemistry, 1943
University of Chicago, Ph.D. Pharmacology-Biochemistry, 1950

Experience:

Professor, Center for Brain Research, University of Rochester, 1965-
Professor, Department of Biochemistry, University of Rochester, 1965-
Professor of Neurophysiology and Biochemistry, U. of Illinois College of Medicine, 1963-65
Director of Research, Department of Psychiatry, U. of Illinois, College of Medicine, 1956-1965
Associate Professor of Neurophysiology and Biochemistry, University of Illinois, College of Medicine, 1954-63
Assistant Professor of Neurophysiology and Biochemistry, University of Illinois, College of Medicine, 1952-54
Instructor in Physiology, University of Chicago, 1950-52

Scientific Societies:

REDACTED

Major Research Interest:

Neurochemistry: Chemistry of Excitation; Neuropharmacology

CURRICULUM VITAE

Karl Lowy

Born:

REDACTED

Education: University of Vienna Medical School, M.D., 1927

Experience:

Professor, Center for Brain Research, University of Rochester, 1960-
Professor, Department of Psychology, University of Rochester, 1968-
Clinical Associate Professor, Department of Otolaryngology, University of Rochester, 1959-
Senior Research Associate, Department of Psychology, University of Rochester, 1943-1968
Research Fellow, Department of Psychology, University of Rochester, 1941-1943
Internship, Medical Arts Center Hospital, New York City, 1938-1939
Instructor, Otolaryngology, University of Vienna, 1935-1938
Internship, Vienna, Allgemeines Krankenhaus, 1929-1930

Licensure, September, 1938, New York State

Scientific societies:

REDACTED

REDACTED

13. Publications

Distribution of piperidine in the brain and its possible significance in behavior. L.G. Abood, Rinaldi and Eagleton, Nature, 191: 201, 1961.

Neuropharmacological investigations on the neural tissue-limb deplant of the salamander. Stuart R. Snider, Leo G. Abood and Ray S. Snider, Expt'l. Brain Res. 6: 81-88, 1968.

The role of proteins and lipids in membrane structure and funtion. L.G. Abood and A. Matsubara, in "Biogenic Amines and Physiological Membranes in Drug Therapy", J.H. Biel and L.G. Abood (eds.), Marcel Dekker, New York, 1971, pp. 1-33.

Interfacial adsorption of a psychotomimetic drug using liquid scintillation. H. Kimizuka and L.G. Abood, J. Pharmaceut. Sci., 62: 740-45, 1973.

Kinetics of Ca^{2+} adsorption and cationic selectivity with a synaptic membrane protein. L.G. Abood and W. Hoss, Biochimica et Biophysica Acta, 332: 85-96, 1974.

Evoked potential and microelectrical analysis of sensory activity within the cerebellum. Ray S. Snider and Karl Lowy, Fourth NASA Symposium on the role of vestibular organs in space exploration. October, 1968

Pulse generator with external amplitude control by computer. K. Lowy and L. Ota, Electroenceph. Clin. Neurophysiol., 25: 389-392, 1968.

Assessing the significance of averaged evoked potentials with an on-line computer: the split-sweep method. K. Lowy and B. Weiss, Electroenceph. Clin. Neurophysiol., 25: 177-180, 1968.

Influence of an anticholinergic psychotomimetic agent on behavior in cats controlled by an auditory stimulus. Karl Lowy, Bernard Weiss, and Leo G. Abood, Neuropharmacology, in press, 1974.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
L. G. Abood K. Lowy	(summer, 3 months)	REDACTED
Technical Laboratory Assistant		REDACTED
	Sub-Total for A	R
B. Consumable supplies (by major categories)		
Animals and animal care		3,000
Chemicals, glassware		500
	Sub-Total for B	3,500
C. Other expenses (itemize)		
Travel		300
Publication costs		200
Service contract on LINC-8		2,000
	Sub-Total for C	2,500
	Running Total of A + B + C	17,500
D. Permanent equipment (itemize)		
	Sub-Total for D	None
	E	2,625
E. Indirect costs (15% of A+B+C)	Total request	20,125

15. Estimated future requirements:

	Salaries	Consumable Suppli.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	12,075	3,500	2,500	None	2,711	20,786
Year 3	12,679	3,500	2,500	None	2,802	21,481

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16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Mechanism of Action of Psychotomimetic Drugs	MH20142	$65,000	6/1/72 - 5/31/75

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name Leo G. Abood

Signature Leo G. Abood Date 5/5/74

Telephone 716 (Area Code) 275-4024 (Number) (Extension)

Checks payable to

Roy B. Thompson, Senior V. Pres. and Treasurer

Mailing address for checks

University of Rochester

Rochester, New York 14627

Responsible officer of institution

Typed Name Richard E. Hufnail

Title Associate Director, Res. & Proj. Admin.

Signature Richard E. Hufnail Date 5/8/74

Telephone 716-275-4034 (Area Code) (Number) (Extension)